IJTA © Serials Publications

National Academy of Agricultural Science (NAAS) Rating : 3.03

"Germination and Seedling Vigour Index" as a Phenotypical Marker for the Selection of NaCI Tolerant in T_3 generation transgenics (PgNHX1) of tomato (*Solanum lycopersicum* L.)

Truong N. N, S. Rangaiah[*] and D. L. Savithramma

ABSTRACT: *The success of increase the solute concentration in the vacuoles of plant cells by sequestering sodium ion into the vacuoles of plant cells, where overexpression of the* Pennisetum glaucum PgNHX1 *that encodes a vacuolar sodium/proton antiporter resulted in higher plant salt tolerance. Percent germination, driven by the endogenous respiration was found to be suitable for sensitive, quantitative differentiation between transformant lines of T3 generation tomato. Salinity tolerance during germination was evaluated for 25 lines of transformant T_3 tomato at 200mM NaCI in* in-vivo *and 100mM NaC1 along with 20mg/L hygromycin in in-vitro. Germination responses were measured as final germination percentage and calculated seedling vigour index. Germination percentage was affected by salinity; control seeds no germinate under NaCI concentration screened. Transformants seeds germinated after 5 days under exposing to 100mM NaC1 in* in-vivo *and 7 days in germination testing in* in-vitro. *Seedling vigour index of transformant lines 4-13/1, 13-7/8, and 4-16/1 screened in* in-vitro *showed high value inturn as 4.03, 3.74, and 3.55 respectively showed salinity tolerance in germination stage even in presence of both salinity and antibiotic stress.*

Keywords: *Tomato, transformants, germination, seedling vigour index, salinity. Introduction*

Salinity in soil is one of the non-living environmental stresses, major limitations of plant growth and productivity all over the world. Thus it is a serious threat to agricultural productivity especially in arid and semi-arid regions [18]. Salt stress is recognized as a major factor that limits plant growth, physiology and productivity, mainly by inducing osmotic effects, ion-specific effects and oxidative stress or plant death [16]. Salinity has negative effects on yield of tomato plants by reducing germination, seedling growth, establishment of the plant weak, lower weight and marketable fruits [10]. Germination is the first life stage of the plant and appearance of radicle is the first easily observable event entirely driven by endogenous respiration. In germinating seeds the energy for growth is provided by respiration. Universal inhibition of growth arises from inhibition of respiration since electron transport (mitochondrial and photosynthetic) in the membrane is inhibited by the inhibition of diffusion of the relevant quinone in the voids in the membrane [12]. The presence of finite maintenance energy requirements necessitates that growth of a plant ceases before the energy source, respiration, does, at any stage in the life cycle. Since the phenotype for osmotolerance was identified with the limiting osmotic pressure at which respiration ceases, and since this critical osmotic pressure could vary with the life cycle, osmotic susceptibility of germination on hydration of the dry seed appeared to be a logical choice for phenotyping plants [13]. Moreover salt stress can affect germination with osmotic pressure [18]. Germination and seedling characteristics, which are the most useful criteria, are used to select the level of salt tolerance in plants [4].

The cultivated tomato (*Solanum lycopersicum* L.) belongs to family *Solanaceace* and is the second most important vegetable crop next to potato. Tomato has the ability to reduce risks of many cancers due to its nutritional content [7]. Salinity has negative effects on yield of tomato plants by reducing germination, seedling growth, establishment of the plant weak, lower weight and marketable fruits [17]. Moreover,

* Department of Genetics and Plant Breeding, UAS, GKVK, Bangalore-560 065, India, *Corresponding author's E-mail: srangaiah@rediffmail.com*

Kaveh *et al.* [10] demonstrated that percentage and speed of germination of all varieties of greenhouse tomatoes delayed and reduced by increasing salinity and whole seedling growth characteristics (fresh and dry weight of roots and stems) were decreased with increasing salinity. Researchers showed that the percentage and speed of germination of all varieties of greenhouse tomatoes delayed and reduced by increasing salinity and whole seedling growth characteristics (fresh and dry weight of roots and sterms) were decreased with increasing salinity.

In this present investigation we have used seed germination rate and final percentage germination phenotypes as the easiest and suitable method to screen salt tolerant lines among transformants T_3 tomato. We have carried this experiment by using 25 lines transformed *(PgNHX1)* gene to Vaibhav variety, and have discussed the validity of seed germination experiment with respect to salt resistant in T_3 generation transformants tomato.

MATERIAL AND METHODS

Material

Seeds of 25 transgenic lines tomato (Vaibhav cultivar overexpressing *PgNHX1* genes) were developed in department of Genetics and Plant breeding, University of Agricultural sciences, Bengaluru. The seeds of transgenic lines after selfing T_2 plants were used for the selection of NaCI tolerant in T_3 generation transgenics *(PgNHX1)* of tomato *(solanum lycopersicum* L). The material used for this study was obtained from the concerned teacher in the department of Genetics and Plant breeding.

Methods

Seed germination assay

First experiment was carried out to calculate germination percentage and seedling vigour index under salt stress in *in-vivo.* Ten seeds from each transgenic line in 25 transformant lines were spreaded on petri plates moistened with distilled water for two days, followed by to 50mM for another two days, continued increasing NaC1 concentration to 200mM. The plates were incubated at 27°C in growth chamber and germination percentage was recorded on 15th day and expressed as percentage over control using the formula.

$$Germination\ percentage\ of\ the\ line = \frac{Germination\ \%\ under\ stress}{Germination\ \%\ of\ related\ control}$$

Another experiment was conducted to assure translation of *PgNHX1* gene in T_3 generation with effect of hygromycin in the presence of NaC1 in *in-vitro.* Ten seeds from each transgenic line in 25 transformant lines were tested for their germination ability at 25°C in presence of 100mM NaCl and 20mg/L Hygromycin supplemented in MS basal medium. Observations were recorded after 15 days of incubation. The seeds which produced a minimum radicle length of 0.5cm were considered to be germinated. The number of seeds that germinated in each bottle was recorded and expressed as percentage over control.

Seedling vigour index

Root and shoot lengths of individual plants were measured 15 days post germination and expressed as percentage over control and seedlings vigour index using the formula.

Seedlings Vigour Index (SVI) = Germination percentage x [shoot length (cm) + root length (cm)]

Experiment design and statistical analysis

Completely Randomized Design (CRD) for all experiments. Germination testing in both of *in-vivo* and *in-vivo* with 27 treatments (25 transformant lines + 2 control treatments), 2 replications, and 5 seeds per replication. Data was input and block diagram used Microsoft® Office Excel 2010, data analysis was used for determining the existence of significant effects due to the transgene *PgNHX1* by IRRISTAT 5.0 software.

RESULTS AND DISCUSSION

Seed germination of PgNHX1 **T3** transgenics and control in 200mM NaC1 in in-vivo and 100mM NaC1 + 20mg/L Hygromycin in in-vitro

Previous studies have also showed that no correlation has been observed between salt tolerance at germination and seedling stage [14], nor between germination and grain yield [3], and between seed germination and in later growth stages [6]; Cuartero and Fernandez-Munoz, [5]). The stimulation of germination and days required for its completion, depend upon Gibberelic acid content in seed. A low level of GA in seed in saline medium was unable to break the mechanical resistance of endosperm against imbibition of water by seed and this leads to the reduction in speed of germination [8]. Furthermore, Naseri *et al.* [15] reported that salinity due to the toxic effects of specific ions and a high concentration of salt reduces the water potential, which prevents the

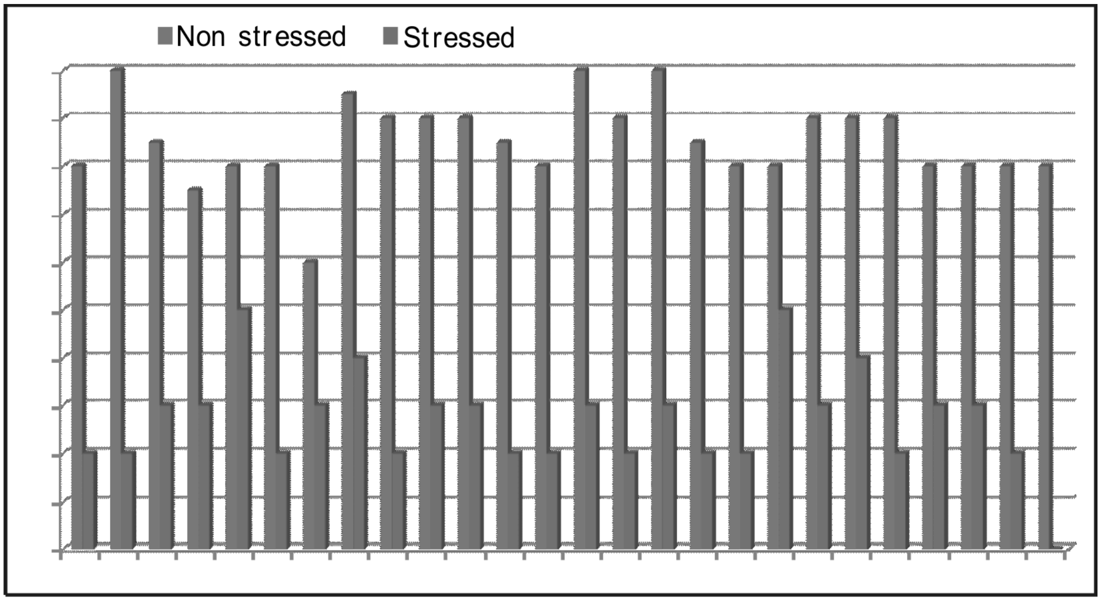

Figure 1: Germination percentage in *in-vitro* of T3 putative transformants and control seeds on exposure to 100mM NaCl along with 20mg/L hygromycin.

absorption of water by the seeds, therefore, reduced the speed of germination. As well as, Sattar *et al.* [18] mentioned that inhibition of seed germination by salinity was related to the osmotic effect or specific ion toxicity.

Genotypes which germinate earlier at higher salinity are supposed tobe more vigourous and may be used as potential donor in salinity tolerance crop breeding programmes [2,9]. In the present study, 25 putative transformant lines were first screened with NaC1 in *in-vivo.* Germination of seeds were started in presence of 100mM NaC1 (at 5th day), the germination percentage reached up to 100 *per cent* in many lines, while the stress control did not germinate. It is also the speed of germination was reduced i.e. it took more days to complete the germination under salinity as Amir *et al.* [2] proposed the salinity notably affects germination in many species but .also lengthens the time needed to complete germination. Moreover, the seedling vigour index was more than 10 in transformant lines and siginificant difference with NaC1 stress control was 0 (no germinate), which was around the value of seedling vigour index of control plant under non-stress (9.05). There are 15 transformant lines showed seedling vigour index more than and significant difference with control non stress at 5%, other transformant lines higher seedling vigour index than control but non significant difference with them (Table 1).

As well as, Akinci *et al. [1]* demonstrated the response of tomato genotypes to increasing salinity during the germination and seedling stages. It has been shown that crops which are tolerant at seedling stage also show improved salinity tolerance at adult stage. Second experiment was conducted to assure translation of *PgNHX1* gene in T_3 generation. Twenty five lines were selected for abiotic resistance screened with 100mM NaC1 along with 20mg/L hygromycin in half strength MS basal media in *in-vitro.* The untransformed seeds failed to germinate while the transgenic lines 4-13/1 and 13-2/8 showed 50 *per cent* seeds germinate.

Moreover, the seedling vigour index of transformant lines 4-13/1, 13-7/8, 4-16/1 showed high value inturn as 4.03, 3.74, and 3.55 respectively. These data showed salinity tolerance in germination stage even in presence of both salinity and antibiotic stress (Table 2). Similarly, the seedling height increased with time but decreased with in creasing salinity level in both cultivars. The development of root system and leaves was reduced as salinity increased. Salt stress caused reduction in the vascuolar system in both roots and stem. Meanwhile, salinity stress resulted in an increase in the thickness of the cortex region of the stem and a reduction in that of root [19].

These two stress treatments of transformant and control seedlings have shown that the transgenics expressed increase seedling tolerance to NaC1 stress as reflected by the larger seedling length compare to the untransformed seedlings. This results are similar with previous studies of Akinci *et al.,* [1]; Amir *et al.,* [2]; Naseri *et al.,* [15]; Mahran, [11].

Table 1
Seedling vigour index of putative transformants and control seedlings after exposing to 200mM NaCI in *in-vivo*

SI. No	Line	SVI	SI. No	Line	SVI
1	4-11/1	11.63'	15	5-28/9	12.36*
2	4-11/2	10.04ns	16	5-28/12	16.05*
3	4-12/3	11.21 ns	17	5-31/2	11.87*
4	4-12/8	11.71 ns	18	13-2/1	12.40*
5	4-13/1	10.15 ns	19	13-2/8	12.75*
6	4-13/3	11.64 ns	20	13-7/4	10.04 ns
7	4-13/4	12.03*	21	13-7/8	15.06*
8	4-16/1	11.55 ns	22	13-7/10	12.22*
9	5-5/7	11.65 ns	23	13-11/4	14.20*
10	5-24/9	14.30*	24	13-11/8	10.46ns
11	5-24/13	15.03*	25	13-11/9	13.19*
12	5-27/2	13.92*	26	Control MSo	9.05
13	5-27/4	11.98*	27	Control NaC1	0.00
14	5-28/2	12.41*			
LSD 5%		2.81			
CV%		11.60			

* significant difference between transformants with control non stress at 5%; ns: non significant

Table 2
Seedling vigour index of putative transformants and control seedlings after exposing to 100mM NaCI along with 20mg/L hygromycin in *in-vitro*

SI. No	Line	SVI	SI. No	Line	SVI
1	**4-11/1**	2.06*	15	5-28/9	1.99*
2	4-11/2	1.67*	16	5-28/12	2.57*
3	4-12/3	2.60*	17	5-31/2	2.13*
4	4-12/8	2.05*	18	13-2/1	2.18*
5	4-13/1	4.03*	19	13-2/8	2.41*
6	4-13/3	2.02*	20	13-7/4	2.22*
7	4-13/4	2.52*	21	13-7/8	3.74*
8	4-16/1	3.55*	22	13-7/10	1.89*
9	5-5/7	1.50*	23	13-11/4	2.48*
10	5-24/9	2.17*	24	13-11/8	2.50*
11	5-24/13	2.10*	25	13-11/9	2.37*
12	5-27/2	1.76*	26	**Control MSo**	9.85
13	5-27/4	1.78*	27	**Control NaC1**	**0.00**
14	5-28/2	2.42*			
LSD 5%		1.19			
CV%		**13.8**			

* significant difference between transformants with control (NaC1 stress) at 5%

CONCLUSION

Some important conclusions can be drawn from the results achieved during this experiment. Tomato is considered to be a salt sensitive species. Our results indicate that *PgNHX1* overexpressing tomato display an enhanced tolerance to NaC1 at early seedling stage. Therefore, comparisons of salinity tolerance mechanisms in transformants tomato will be fundamental to understand the regulatory points assigning physiological mechanisms that are likely to provide salinity tolerance.

ACKNOWLEDGMENT

The authors are grateful to Department of Genetics and Plant breeding, Agriculture College, GKVK and Plant Biotechnology department for providing facilities to carry out the present investigations.

REFERENCES

Akinci, I. E., Akinci, S., Dikici, I. H. K., (2004), Response of eggplant varieties *(Solanum melongena)* to salinity in germination and seedling stages. *New Zealand J. Crop. Hort. Sci.,* 32: 193-200.

Amir, N., Muhamad, A., Muhamad, A. P., Irfan, A., (2011), Effect of halopriming on germination and seed ling vigor of tomato. *Afr. J. Agr.,* **6** (15): 3551-3559.

Ashraf, M., and T. McNeilly., (1988), Variability in salt tolerance of nine spring wheat cultivars. *J. Agron. Crop Sci.,* **160:** 14-21.

Boubaker, M., (1996), Salt tolerance of durum wheat cultivars during germination and early seedling growth. *Agric. Medit.,* **126:** 32-39.

Cuartero, J., and Fernandez-Munoz, R., (1999), Tomato and salinity. *Sciencia Horticulturae,* **78:** 83-125.

Foolad, M. R., and Lin, G. Y., (1997), Genetic potential for salt tolerance during germination in *Lycopersicon species. Hort. Science,* **32:** 296-300.

Foolad, M. R., (2004), Recent advances in genetics of salt tolerance in tomato. *Plant Cell Tiss. Org.,* **76:** 101-119.

Groot, S. P. C., Karssen, C. M., (1992), Dormancy and germination of abcissic acid deficient tomato seeds. *Plant Physiol.,* **99:** 952-958.

Hamed, K., Hossein, N., Mohammad, F., Safieh, V. J., (2011), How salinity affect germination and emergence of tomato lines. *J. Biol. Environ. Sci.,* **5 (15):** 159-163.

Kaveh, H., Nemati, **H.,** Farsi, M., Jartoodeh, S. V., (2011), How salinity affect germination and emergence of tomato lines. *1 Biol. Env. Sci., 5:* 159-163.

Mahran, M. Elnagar., (2013), Genetic engineering for increasing salinity tolerance in tomato. *Australian Journal of Basic and Applied Sciences,* **7 (1):** 433-440.

Mathai, J. C., Sauna, Z. E., John, **0.,** and Sitaramam, V., (1993), Rate limiting step in electron transport: osmotically sensitive diffusion of quinones through voids in the bilayer. *J. Biol. Chem.,* **268:** 15442-15454.

Munns, R., (2002), Comparative physiology of salt and water stress. *Plant, Cell and Environment,* **25:** 239-250.

Munns, R., James, R. A., (2003), Screening methods for salt tolerance: A case study with tetraploid wheat. *Plant Soil,* **253:** 239-250.

Naseri, R., Mirzaei, A., Emami, T., Vafa, P., (2012), Effect of salinity on germination stage of rapeseed cultivars *(Brassica napus* L.). *J. Agri. Crop Sci.,* **4 (13):** 918-922.

Okhovatian-Ardakani, A. R., Mehrabanian, M., Dehghani, F., Ak-barz.adeh, A., (2010), Salt tolerance evaluation and relative comparison in cuttings of different pomegranate cultivars. *Plant, Soil and Environment*, **56:** 176-185.

Sato, S., Sakaguchi, S., Furukawa, **H.,** and Ikeda, H., (2006), Effects of NaCl application to hydroponic nutrient solution on fruit characteristics of tomato *(Lycopersicon esculentum* Mill.). *Sci. Hortic.*, **109:** 248-253.

Sattar, S., Hussnain, T., Javaid, A., (2010), Effect of NaC1 salinity on cotton grown on MS and in hydreoponic culture. *J. Animal and Plant Sciences*, **20 (2):** 87-89.

Sharaf, AT., and Iraki, N., (2013), Morphological and anatomical responses of two Palestinian tomato *(Solanum lycopersicon* L.) cultivars to salinity during seed germination and early growth stages. *African Journal of Biotechnology*, **12 (30):** 4788-4797.